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FLUOROMETRIC TECHNIQUES FOR TRACING RESERVOIR INFLOWS

by

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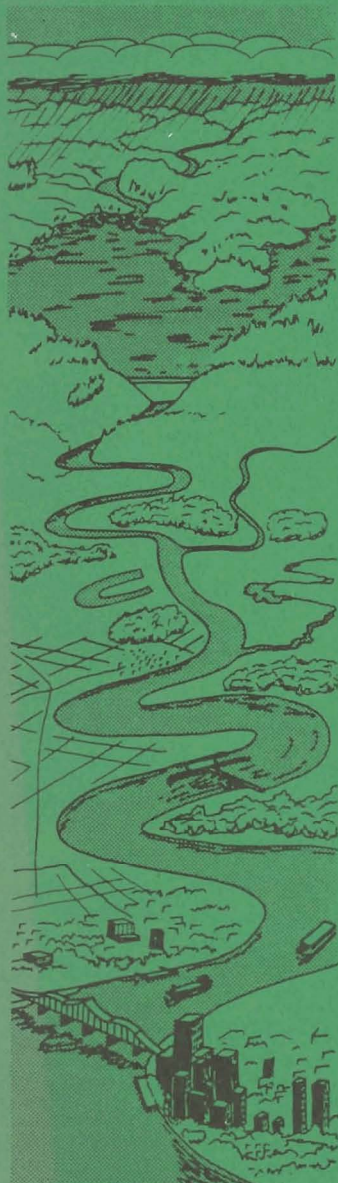
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    A reservoir's storage capacity, trophic state, and water quality are affected by tributary inflows which transport suspended and dissolved materials into and through the reservoir. The impact these materials have on reservoir and release water quality depends on how the inflow moves through and mixes within the reservoir. The movement of the inflow in a reservoir is often influenced by such factors as irregular morphometry, variable

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20. ABSTRACT (Continued).

stratification, and engineering activities (e.g., preimpoundment clearing of trees, submerged cofferdams, and project operation).

A reliable means of determining how inflows move and mix within a reservoir is to conduct field investigations using a fluorescent dye as a tracer.

This report presents a methodology for conducting such a study. Included is information on the characteristics of fluorescent dyes, the principles and equipment of field fluorometry, methods for properly marking an inflow with dye, sampling methods, and guidance on data analysis.

PREFACE

This report was prepared as part of the Environmental and Water Quality Operational Studies (EWQOS) Program, Work Unit IA, Develop and Verify Techniques for Describing Inflow Mixing Processes. The EWQOS Program is sponsored by the Office, Chief of Engineers (OCE), and is assigned to the U. S. Army Engineer Waterways Experiment Station (WES), under the purview of the Environmental Laboratory (EL). OCE Technical Monitors were Mr. Earl Eiker, Dr. John Bushman, and Mr. James L. Gottesman. Dr. J. L. Mahloch, EL, is the Program Manager of EWQOS.

This report was written by Mr. Marc C. Johnson, Water Quality Modeling Group (WQMG), Ecosystem Research and Simulation Division (ERSD), EL, who conducted and participated in several reservoir inflow tracer studies as part of EWQOS Task IA. This report was reviewed by Mr. Mark S. Dortch, Chief, WQMG. Mr. Johnson worked under the general supervision of Mr. D. L. Robey, Chief, ERSD, and Dr. John Harrison, Chief, EL.

The following individuals provided valuable comments and suggestions throughout the conduct of this study: Dr. Dennis Ford and Dr. Kent Thornton of Ford, Thornton, Norton and Associates, Ltd.; Ms. Linda Johnson, Mr. Jack Waide, Mr. Aaron Stein, and Mr. Theodore Kientz, WQMG; Mr. Jeff Leighton, Cornell University; and Mr. Gene Parker, U. S. Geological Survey, Water Resources Division, Maine District.

The Commander and Director of WES during the preparation and publication of this report was COL Tilford C. Creel, CE. The Technical Director was Mr. F. R. Brown.

This report should be cited as follows:

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CONTENTS

FLUOROMETRIC TECHNIQUES FOR TRACING RESERVOIR INFLOWS

PART I: INTRODUCTION

1. Inflows affect a reservoir's storage capacity, trophic state, and water quality through the transport of suspended and dissolved materials. The fate of these materials and their impact on reservoir and release water quality depend on how the inflow moves through and mixes within the reservoir. Analysis of these processes is often complicated by such factors as irregular morphometry, variable stratification, and man-made obstructions (Ford and Johnson 1983). However, inflow field studies can be used to determine how inflows move through and mix within a reservoir.

2. A variety of techniques have been used in inflow field studies. Elder and Wunderlich (1972) describe the use of a sensitive current meter to monitor inflow movements in a reservoir. These measurements provided excellent data but were time-consuming, which ultimately limited their usefulness.

3. A technique that allows much more data to be collected involves tracking the naturally occurring inflow suspended or dissolved materials through the reservoir. Measurements of salinity, turbidity, temperature, and suspended or dissolved materials have been reported (Grover and Howard 1938; Fry, Churchill, and Elder 1953; Churchill 1958; Elder and Wunderlich 1972; Serruya 1974; Hebbert et al. 1979; Ford and Johnson 1980). Natural tracers, however, are often nonconservative or unique to a given location, which limits their general applicability.

4. When natural tracers have been unavailable, artificial tracers have been introduced to mark the inflow. Radioactive tracers have proved to be effective but have been used only sparingly since their use involves special handling and safety precautions and requires special approval and licensing. Commercially produced fluorescent dyes have been easier to handle and have been used extensively in inflow studies (Bayne 1967; Elder and Wunderlich 1968; Lawrence 1969; Morris and Thackston

1969; Chasse and Slotta 1972; Fischer and Smith 1983). Many of these dyes, however, are poor tracers due to their low resistance to adsorption, rapid decay, or high toxicity.

5. As a result of these problems, a fluorescent dye, Rhodamine WT, was developed specifically for water tracing purposes. Rhodamine WT has proven its utility in numerous inflow studies (Bayne 1967; Lawrence 1969; Chasse and Slotta 1972; Ford and Johnson 1980; Sutron Corporation 1980; Parker and Hunt 1983; Kennedy, Gunkel, and Carlile 1983; Fischer and Smith 1983).

6. Guidance for conducting an inflow field study using a fluorescent dye such as Rhodamine WT has been lacking. The purpose of this paper, therefore, is to describe how to perform such a study. Only a general methodology can be presented, however, since each study will have a unique set of objectives. The simplicity of the methods presented allows the flexibility needed for each study's unique and often dynamic set of conditions. This paper also serves to update the information on dyes, techniques, and equipment presented in the widely referenced general treatise on fluorometric analysis by Wilson (1968). Also, it should be noted that many of the fluorometric techniques used in marking and following a tributary inflow can be adapted for use in other types of reservoir studies (e.g., cove exchange, hydropower reaeration, time of travel, and dispersion) and in other environments such as rivers, lakes, and cooling ponds as well, providing one considers the assumptions upon which the methods were based.

PART II: FLUORESCENT DYES AND WATER TRACING

7. Strongly fluorescent materials such as the dyes used in water tracing are unique in that they efficiently convert absorbed light into emitted light in a characteristic pair of spectra. With the proper light source and filter combination, a fluorometer can measure the amount of a given fluorescent material in a sample. Thus, when a fluorescent dye is mixed with a given parcel of water, that parcel may be identified and followed as it moves within another body of water. The amount of mixing that takes place between the dyed parcel and ambient water may also be quantified by observing reductions in dye concentrations.

8. Fluorescent dyes have been used in water tracing since 1887 when fluorescein, a green fluorescent dye, was chosen as a tracer for groundwater studies in France (Dole 1906). Extensive use of fluorescent dyes as tracers did not begin until the late 1950's, however, when scientists at the Chesapeake Bay Institute and Japanese Atomic Energy Research Institute independently began to examine the use of dyes in quantitative turbulent diffusion studies (Carter and Okubo 1978). Both groups chose Rhodamine B, a commercially available orange dye that was, unlike fluorescein, stable in sunlight. It was soon learned that Rhodamine B had a major flaw as a tracer dye; it was readily adsorbed to sediments, making quantitative analyses difficult. As a result, in the mid-1960's E. I. Dupont Nemours developed a dye specifically for water tracing, Rhodamine WT (Carter and Okubo 1978).

9. Rhodamine WT was determined to be the most satisfactory dye for water tracing by Smart and Laidlaw (1977) in a comprehensive evaluation of eight fluorescent dyes: Fluorescein, Rhodamine B, Rhodamine WT, Sulpho Rhodamine B, Lissamine FF, Pyranine, Amino G Acid, and Photine CU. As a result of this recommendation, the author's experience, and the results reported by others (as referenced in paragraphs 2-5), Rhodamine WT has been chosen as the dye most suitable for use in inflow studies and is the one that will be discussed throughout this paper. For a detailed discussion of the other dyes previously mentioned, the reader is referred to Smart and Laidlaw (1977).

PART III: RHODAMINE WT CHARACTERISTICS

Convenience and Economy

10. Rhodamine WT is produced commercially as a 20-percent solution (by weight) of dye in water and solvents with a specific gravity of approximately 1.2. Powdered forms of dye are often awkward to handle and can be difficult to dissolve. Rhodamine WT can be obtained in a variety of prepackaged quantities, making handling even easier. Although Rhodamine WT is one of the more expensive fluorescent dyes, its low minimum detection limit (0.01 ppb) and conservative nature make it one of the most economical to use since a large volume of water can be marked with a small quantity of dye.

Chemical and Photochemical Decay

11. For conditions typically found in natural waters, no significant loss of Rhodamine WT is likely to occur due to chemical or photochemical decay. In fact, the dye's conservative nature prompted the U. S. Geological Survey (USGS) to recommend that Rhodamine WT be used for any study requiring a high recovery of dye (Wilson 1968).

12. Deaner (1973) found that chlorine caused significant losses of the dye during long exposures (20 hours) at high residual chlorine concentrations (greater than 9 mg/ℓ). At low concentrations, however, only minor losses occurred (5 percent at residual concentrations less than 5 mg/ℓ for exposures of 20 hours). Thus, the discharge of chlorinated water into an area where the dye exists should cause no harm unless the chlorine residuals are unusually high.

13. Photochemical decay, unfortunately, is much more difficult to quantify. The rate of photochemical decay depends not only on the dye concentration present but also on the intensity of light reaching the dye. In the water column of a natural system, light intensity is influenced by many factors, including cloud cover, water turbidity, and water column depth. Consequently, it is difficult to use the decay

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rate observed from one study to predict photochemical decay for another. Smart and Laidlaw (1977) incorrectly reported values from Abood, Lawler, and Disco (1969), citing several photochemical decay rates when in fact a total decay rate of 0.04/day had been determined. This included losses due to both photochemical decay and sorption.

14. Given the relatively small total decay rate of 0.04/day reported by Abood, Lawler, and Disco (1969) and the low photochemical decay rates reported for other Rhodamine dyes (Pritchard and Carpenter 1960; Feuerstein and Selleck 1963; Watt 1965), it may be assumed that losses due to the photochemical decay of Rhodamine WT in a natural environment are negligible.

Effects of pH, Temperature, and Salinity

15. Smart and Laidlaw (1977) found that pH, temperature, and saline conditions have the greatest influence on the fluorescence of Rhodamine WT. Above a pH of 5.5, adsorption of the anionic Rhodamine WT to the negatively charged surface of organic materials is retarded; below 5.5, however, the carboxyl acid group in the dye molecule becomes protonated, allowing adsorption to occur more freely. Protonation of the acid group may also cause a resonance change in the molecule, resulting in a reduction in fluorescence.

16. Smart and Laidlaw (1977) reported 18 percent and 89 percent adsorption losses in humic sediment solutions of 2.0 g/ℓ and 20.0 g/ℓ, respectively, with 100 ppb dye concentrations. These results were not unexpected since the increase in humic content caused both a reduction in pH below the critical value of 5.5 and an increase in the surface area available for adsorption. In contrast, only 11 percent and 23 percent losses were observed with similar solutions of Kaolinite clay.

17. Smart and Laidlaw (1977) also quantified the change in intensity of Rhodamine WT fluorescence due to temperature variations. They formulated a relationship between the two which can be expressed as:

$$F = F_o \exp [-0.027 (T - T_o)] \tag{1}$$

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where

F = fluorescence at temperature T (°C)

F_o = fluorescence measured at calibration

T_o = temperature of solution at calibration (°C)

A rule of thumb derived from Equation 1 is that fluorescence decreases 5 percent for every 2° C increase in temperature.

18.   Another factor that may impact the fluorescence of Rhodamine WT is salinity. In their tests on the effects of potassium and sodium chloride solutions on the dye, Smart and Laidlaw (1977) found a slow but significant decay of the dye in solution of potassium chloride greater than 0.002 molarity (M) and in solutions of sodium chloride greater than 0.02 M. Thus, losses in saline environments previously reported (e.g., Yotsukura and Kilpatrick 1973) and attributed to adsorption to sediment may actually have been due to salinity effects.

Toxicity

19.   Rhodamine WT has generally been thought to have a very low toxicity level. Parker (1973) showed that Rhodamine WT had no harmful effects on the development of silver salmon, Donaldson trout, and Pacific oyster eggs. Smart and Laidlaw (1977) also cite the unpublished work of Donaldson who reported no traumatic effects for subcutaneous injections or oral feedings of the dye in laboratory rats. The U. S. Food and Drug Administration, however, issued a policy statement on 22 April 1966 restricting ingestion limits for Rhodamine WT to 0.75 mg/ℓ/day. The USGS has set even stricter standards for its field applications, limiting concentrations near water intakes to 10 ppb (Wilson 1968).

20.   In a recent paper, Abidi (1982) reported results of laboratory tests which showed that Rhodamine WT can react with nitrites to form diethylnitrosamine, a known animal carcinogen. However, the study was conducted using very high nitrite concentrations at levels unlikely to be found in natural waters. Moreover, it is unlikely that nitrite-rich waters would be used as a drinking water supply since nitrites themselves can pose a health hazard when sufficiently concentrated. The

Criteria and Standards Division, Office of Drinking Water, of the U. S. Environmental Protection Agency has not issued any regulations on the use of Rhodamine WT nor has it placed any restrictions on its use, pointing out that (in addition to such waters being unsuitable for drinking water) such waters could also contain other secondary amines which might form nitrosamines.* Therefore, before using Rhodamine WT in any field study, it is advised that the proper local and state authorities be consulted to determine what requirements must be met prior to and during its use (e.g., preparing impact statements based on knowledge of nitrite concentrations in the application area or limiting concentrations near water intakes to less than 10 ppb).

Rhodamine WT and Lissamine FF

21. In some instances it may be desirable to use two different fluorescent dyes simultaneously. This might permit conducting two studies simultaneously (e.g., to conduct a study on a second tributary inflow or to make a second release on the same tributary inflow to compare its behavior in the reservoir with that of a previous release). Under such a scenario, Lissamine FF could be used since it fluoresces at a different wavelength. When compared to Rhodamine WT, Lissamine FF is less effective overall as a tracer; however, it does possess some desirable traits.

22. One of the reasons why Lissamine FF is not widely used is that it is expensive. Lissamine FF is nearly twice as expensive per pound as Rhodamine WT. In addition, much more dye is required per application to mark a given parcel of water because it fluoresces in a band where high background levels exist in natural waters and thus is not as strongly fluorescent as Rhodamine WT (Smart and Laidlaw 1977).

23. Another disadvantage in the use of Lissamine FF is that little is known about its toxicity. In fact, no data are known to be

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* Personal Communication, 1983, E. Bellack, Special Assistant for Chemistry, Criteria, and Standards Division, U. S. Environmental Protection Agency, Washington, D. C.

published on its toxicity. Thus, the substitution of Lissamine FF for Rhodamine WT for toxicity reasons is questionable.

24. Despite its drawbacks, Lissamine FF does have several good traits. It is more resistant to adsorption than Rhodamine WT, is stable at low pH, and its fluorescence is almost uninfluenced by temperature differences in the range of 5° to 40° C.

25. In one of the few known applications of Lissamine FF in an inflow study, Ford and Thornton (1983) described its performance as "adequate." They noted that the lack of temperature influence on fluorescence was advantageous when working in a thermally stratified reservoir but, overall, found that the disadvantages outweighed the advantages. In addition to the disadvantages mentioned previously, Ford and Thornton also noted that the dye (a) was extremely unstable in the presence of low chlorine residuals (1.0 and 5.25 mg/ℓ residual chlorine concentrations reduced a 100 ppb solution of Lissamine FF to 50 and 14 ppb, respectively, in less than one hour); (b) had a minimum detectability two orders of magnitude higher than that of Rhodamine WT (1 ppb); and (c) had a significantly lower solubility than indicated by the manufacturer (0.2 mg/ℓ versus 0.4-0.6 mg/ℓ). Thus, Lissamine FF can be used, but its overall performance as a tracer may be inferior to that of Rhodamine WT.

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Theory of Operation

26.   Unlike sophisticated and complex analytical laboratory spectrofluorometers, filter fluorometers are relatively simple instruments. Basically, filter fluorometers are composed of six parts: a light (excitation energy) source, a primary or "excitation" filter, a sample compartment, a secondary or "emittance" filter, a photomultiplier, and a readout device.

27.   When a fluorescent material is placed in a fluorometer, that spectral portion of the light source which coincides with the known excitation spectrum of the test material is allowed to pass through the primary filter to the sample chamber. This energy is absorbed by the fluorescent material, causing electrons to be excited to higher energy levels. In returning to its ground state, the fluorescent material emits light that is always at a longer wavelength and lower frequency than the light that was absorbed. It is this property that is the basis of fluorometry, the existence of a unique pair of excitation and emission spectra for different fluorescent materials. Finally, only a certain band of the emitted light is passed through the secondary filter to the photomultiplier where a readout device indicates the relative intensity of the light reaching it. Thus, with different light sources and filter combinations, the fluorometer can discriminate between different fluorescent materials.

28.   The selection of light sources and filters is crucial since they determine the sensitivity and selectivity of the analysis. Fluorometer manufacturers recommend and supply lamps and filters for most applications, including Rhodamine WT applications.

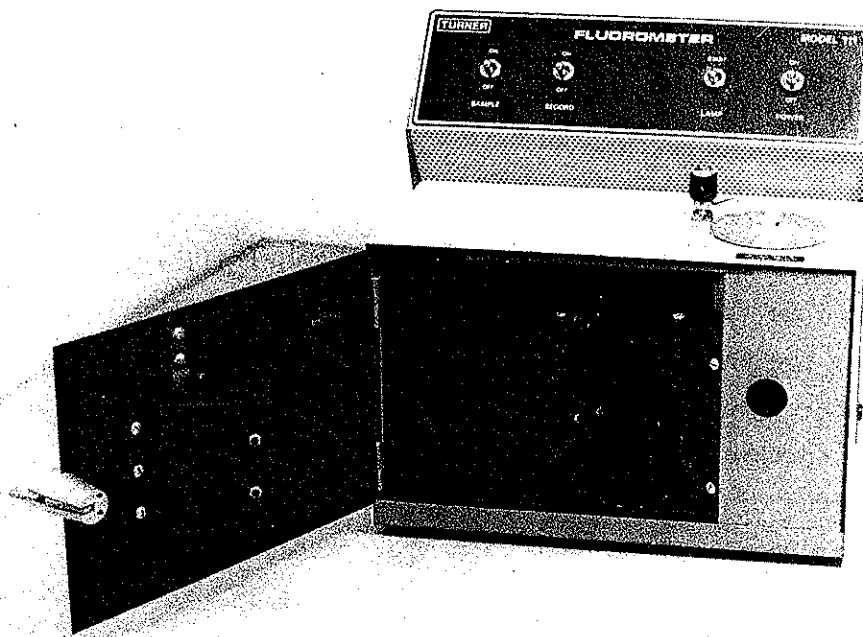## Turner Model 111 and Turner Designs
Model 10 Fluorometers

29.   Two types of fluorometers are in common field use today. The standard instrument used in water tracing by many groups, including the

USGS (Wilson 1968), has been the Turner Model 111 manufactured by G. K. Turner Associates (Figure 1a). Turner Designs has capitalized on recent advances in electronics and optics and developed a fluorometer, the Model 10 series (Figure 1b), that is better adapted to field use than the Turner Model 111. Beyond the fact that both instruments are filter fluorometers, few similarities exist between the two.

30. Differences between the Turner Designs and Turner Model 111 fluorometers begin with their power requirements. The Turner Designs fluorometer is operable from 117-volt and 230-volt AC sources and 12-volt DC sources. (A fully charged deep-cycle, 12-volt marine battery will power a Turner Designs fluorometer for up to 10 hr.) The Turner Model 111 fluorometer can be operated only from 112-volt AC sources, meaning that a generator is required for its use in the field.

31. Once connected to a power supply, both models require a warmup period to bring their optical components to an equilibrium temperature to ensure constancy of output. Kilpatrick, Sayre, and Richardson (1967) warn that the manufacturer-recommended 2-min warmup period for the Turner Model 111 is insufficient and suggest that a 2-hr period be allowed instead. The Turner Designs fluorometer, on the other hand, requires only a 30-min warmup period.

32. The Turner Model 111 and Turner Designs fluorometers also differ in the way their sensitivities are changed. With the Turner Designs fluorometer the sensitivity is changed up to an order of magnitude by altering the relative positions of two polarizing filters or, in a gross adjustment, by using a reference filter. The position of the polarizing filters can be controlled either manually or automatically by the instrument itself. When in the latter mode, the position of one of the polarizers is automatically changed to alter the sensitivity when the instrument's meter needle is either beyond full scale or falls below 20 percent of full scale deflection. This feature is especially convenient since it allows the fluorometer to be left unattended when operated in flow-through mode with some types of chart recorders, even in a location where concentrations may vary over several orders of magnitude. The sensitivity of the Turner Designs fluorometer can also be changed by

a. Turner Model 111 equipped for analyzing discrete samples.
(Cuvettes are inserted in sample chamber in door.)
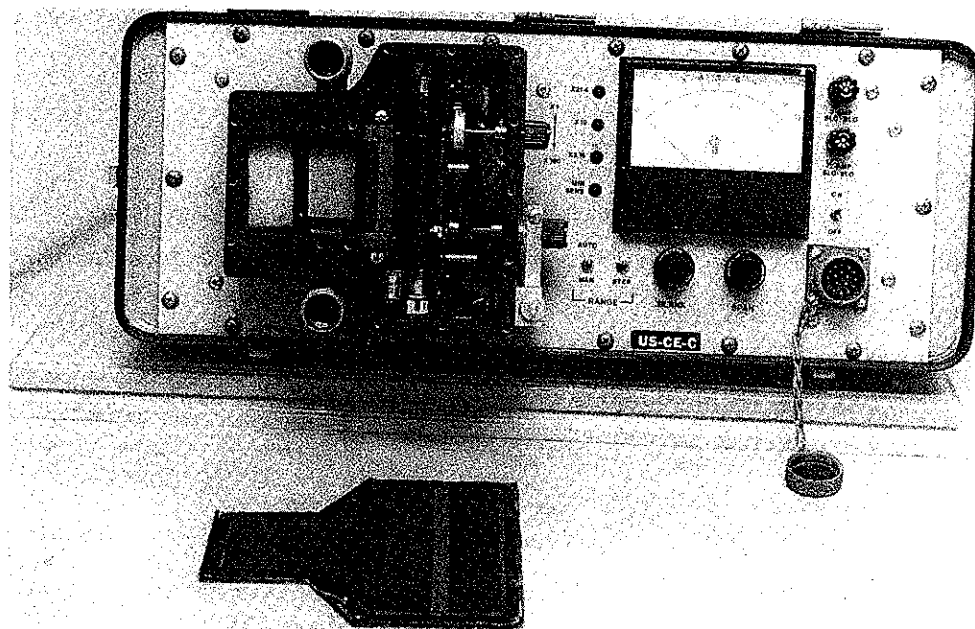
b. Turner Designs Model 10 with sample chamber cover removed.
The two ports above and below the left half of the sample
chamber are the exhaust and intake manifolds, respectively,
that are used during flow-through operation.

Figure 1. Two filter fluorometers used for water tracing

manually adjusting the position of a reference filter. When the reference filter is moved from the light path, the sensitivity is increased two orders of magnitude. Thus, by manipulating the reference filter and polarizers, the Turner Designs fluorometer can be changed over three orders of magnitude.

33. The sensitivity of the Turner Model 111 fluorometer can also be changed in two ways. To change up to two orders of magnitude in sensitivity, the sample compartment door must be opened to select one of four different apertures that control the amount of light passing from the lamp to the primary filter. To increase the sensitivity an additional order of magnitude (equaling that of the maximum sensitivity of the Turner Designs fluorometer), an accessory door with a specially designed sample chamber must be installed. It is recommended that this accessory be used since the increase in sensitivity will mean that less dye will be required for a given application. This is extremely desirable when an expensive dye such as Rhodamine WT is used or when concentrations must be limited near water intakes.

Calibration

34. In order to compare fluorescence readings between fluorometers and to determine the corresponding dye concentration value from the readings, fluorometers must be calibrated.

Preparation of standards

35. Calibration involves the use of standard solutions (prepared solutions of known concentration) which are diluted from a stock concentrate according to a scheme similar to the example given in Figure 2. In general, at least four or five standard solutions are prepared over a range determined by the maximum and minimum concentrations expected in field samples.

36. The preparation of Rhodamine WT dye standards begins with the stock solution which is a 20-percent solution of dye in water and solvents. The first dilution is a particularly formidable task because of the stock solution's viscous nature and dark color, which make it

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Rhodamine WT dye is 20% by weight in stock solution with a
specific gravity of 1.2 or has a concentration of
2.4×10^8 ppb.

Stock
Solution

10 ml Stock (10 g)

C_A 2.4×10^6 ppb

10 ml C_A

C_B 2.4×10^4 ppb

20 ml C_B

1   480 ppb

500 ml 1   150 ml 1   50 ml 1   250 ml 1

2 240 ppb   3 72 ppb   4 24 ppb   5

120 ppb

200 ml 3   40 ml 3   20 ml 4   5 ml 4

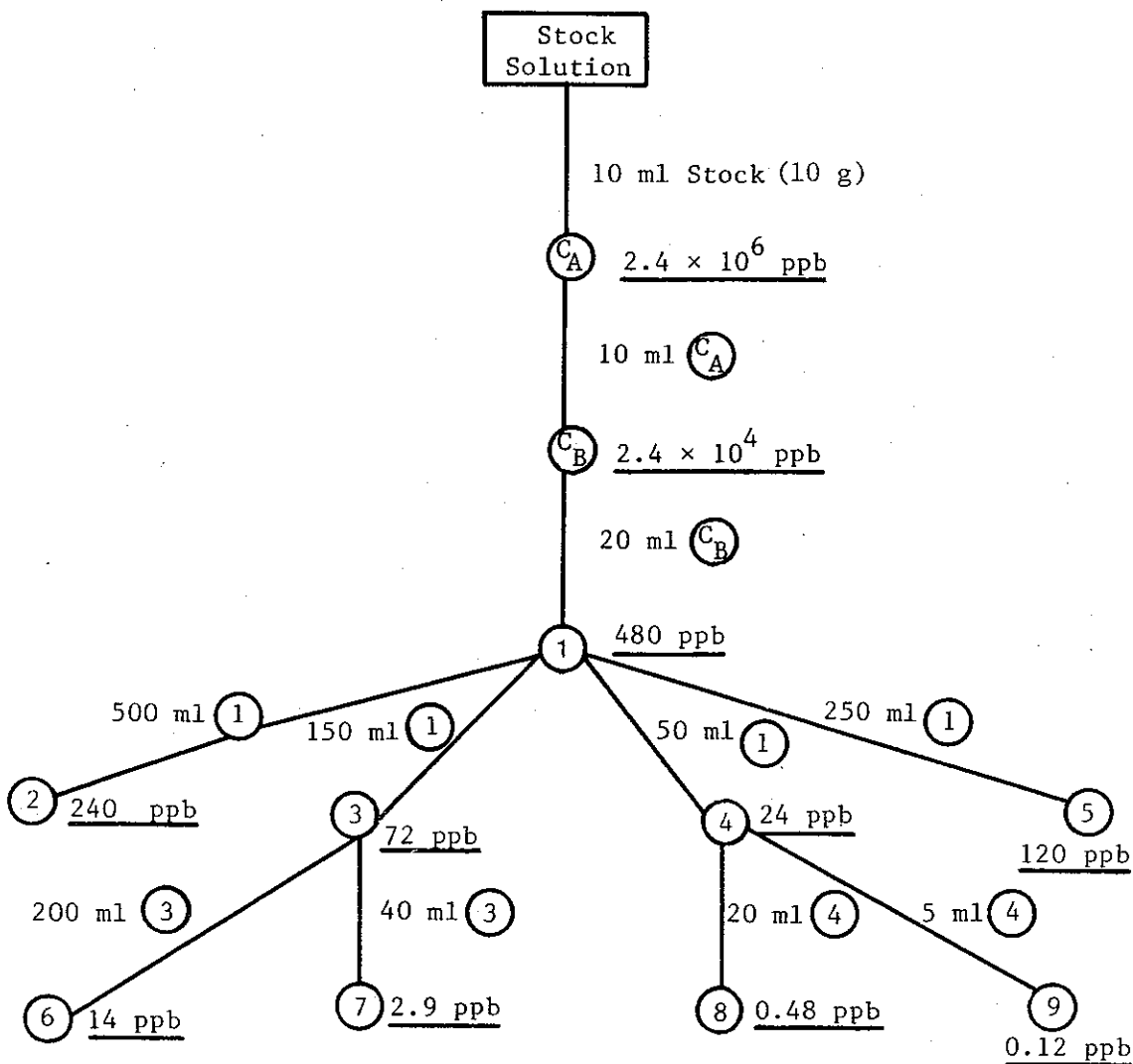6 14 ppb   7 2.9 ppb   8 0.48 ppb   9

0.12 ppb

Figure 2.   A sample dilution scheme for the preparation of Rho-
damine WT standards.   The quantity along each path line is the
volume of the given solution that is required to make the next
dilution.   This volume is placed in a 1-ℓ volumetric flask;
distilled water is then added to fill the flask and bring the
solution to the proper concentration.

difficult to extract and measure. The process begins by carefully weighing out a small amount of dye (e.g., 10 g) in a volumetric container. The dye is then diluted with distilled water. Subsequent dilutions can then be made with pipettes.

37. In order to create an accurate set of standard solutions, good laboratory technique must be used. Dilutions must be made accurately since any errors are propagated down the dilution chain. When making the dilutions, ordinary tap water should never be used. Tap water contains a chlorine residual that may be strong enough to affect the dye. Also, each solution must be well mixed prior to the start of the next dilution. Mixing should be done gently; the first few dilution solutions will tend to foam when violently mixed, making it difficult to accurately fill the volumetric flask.

38. When the dilutions are completed, the standards can be stored in either glass or plastic containers, but must be sheltered from light since prolonged exposure (i.e., over a period of months) may cause photochemical decay.

Calibrating the fluorometer

39. Once the standards are prepared, the calibration of the fluorometer may begin. The calibration procedure always begins with the recording of the temperature of the standards. This information is needed for correcting the values of fluorescence obtained from samples at other temperatures (Equation 1). Then, when the fluorometer is set up for discrete sampling analysis, a cuvette is filled with a standard solution, wiped clean and dry to prevent optical interference, and inserted into the sample chamber for analysis.

40. When the fluorometer is to be used in the flow-through mode, it is necessary to have either a large volume of each standard to circulate through the system or to attach special plumbing to the intake and exhaust manifolds (i.e., short pieces of opaque hose or tubing) to allow the standard solution to fill the sample chamber. The sample chamber should always be filled from the bottom up to ensure that it is free of trapped air bubbles.

41. The next step in the calibration procedure is to test each

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standard on each sensitivity (range) setting. Then the relationship between fluorometer readout and dye concentration can be determined. Figure 3 shows this relationship for four sensitivity settings using a Turner Model 111 fluorometer and six standard solutions; what is not shown in this figure is that when Rhodamine WT concentrations exceed 100 ppb, the relationship becomes nonlinear due to quenching of the excitation energy in the sample. When quenching occurs (i.e., the relationship becomes nonlinear), samples should be diluted to bring their concentrations within the linear range of the calibration curve.
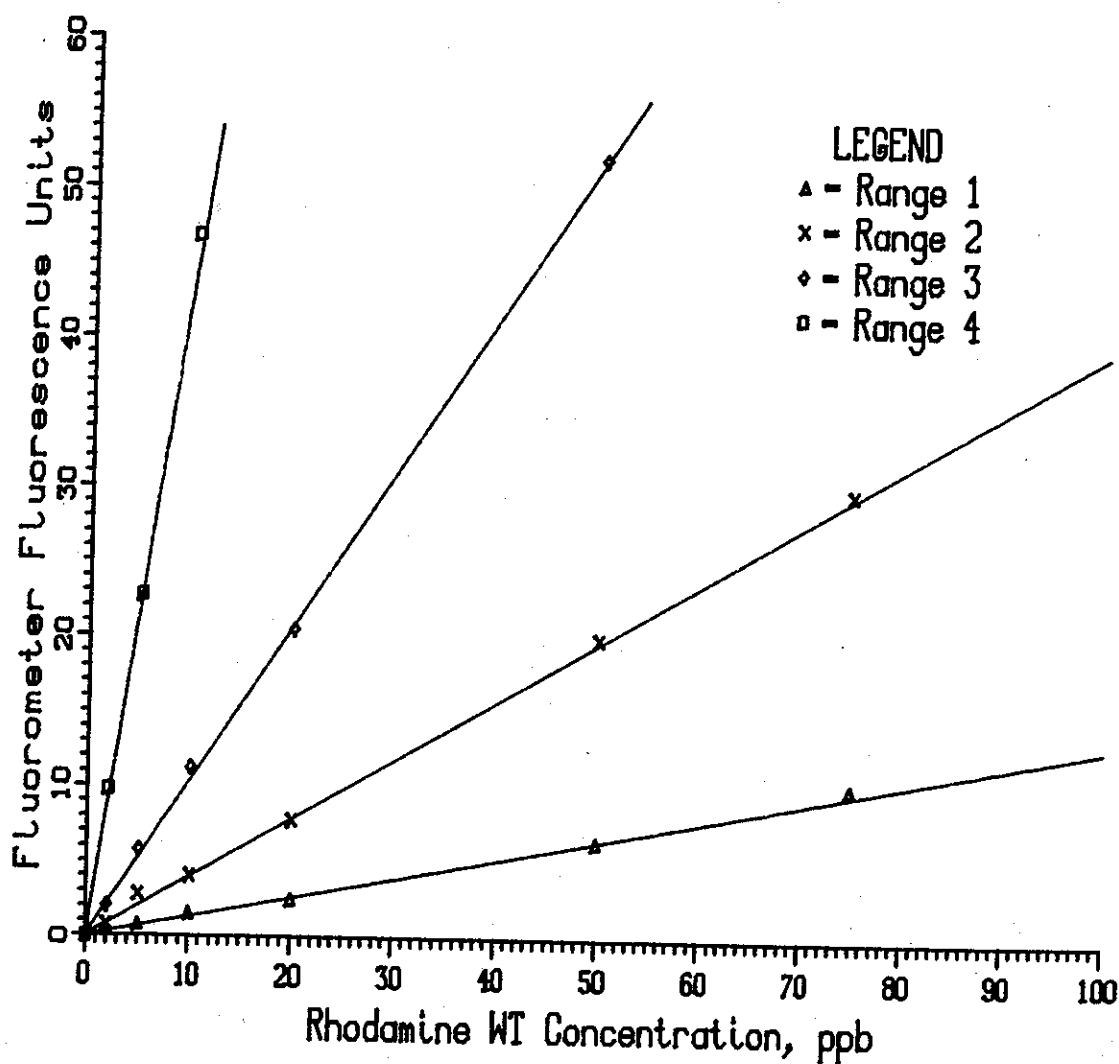

Figure 3. An example of Turner Model 111 fluorometer calibration results for all four sensitivity ranges.

42. The Turner Designs fluorometer can be calibrated to output concentration units directly, provided background fluorescence does not vary. If background fluorescence does not vary throughout the study area, it may be compensated for by zeroing the fluorometer readout on a representative background sample. Then, a standard is inserted into the sample chamber and the fluorometer output is adjusted to read as desired. Concentrations can then be read directly as long as they remain below the quenching level (approximately 100 ppb for Rhodamine WT). If quenching occurs, the sample may be diluted to bring its concentration below this level.

Field Use

43. Once a fluorometer is calibrated, it must be decided where and how field samples will be analyzed--in situ or in a laboratory, continuously or discretely. During in situ analysis, the operation of the fluorometer in flow-through mode (where water from a given location in the water body is pumped continuously through the sample chamber in the fluorometer) is advantageous over its operation in cuvette mode (where a discrete sample is analyzed). Specifically, in situ flow-through analysis (a) allows the homogeneity of fluorescence in the water body to be easily observed, (b) eliminates the need for handling individual samples, and (c) for time of travel and dispersion studies, permits good definition of both spatial and temporal concentration distributions (concentration versus distance from a reference point at a specific point in time, and concentration versus time at a specific location, respectively). Also during in situ flow-through analysis, a strip chart recorder can be attached to the fluorometer, simplifying data collection by providing a continuous record of the fluorescence measured. During laboratory analysis, however, the flow-through system is seldom used since discrete samples are homogeneous and usually lack the volume needed to fill the system. Instead, the fluorometer is operated in cuvette mode where only a small portion of a sample is required for analysis.

44. Each method of analysis also has its inherent problems. Laboratory analysis requires that discrete samples be collected, bottled, labeled, stored in the field, and then transported to the laboratory; this introduces many opportunities for samples to be lost through mislabeling, misplacement, or breakage. Also, when samples are analyzed in the laboratory, sampling locations are usually predetermined. Often, sample analysis shows that the sampling effort was insufficient in some locations.

45. In situ analysis, on the other hand, is usually performed under adverse environmental conditions--often at a fast pace, in a cramped and unsteady work space, or in less than ideal weather conditions. Thus, it is more likely that an error will occur during in situ analysis than during analysis in the controlled environment of a laboratory. It is also usually necessary to compute and apply many more temperature correction factors to fluorescence values during in situ analysis than during a laboratory analysis, since the samples to be analyzed in situ have not had a chance to reach a common temperature. This also increases the chances for error during analysis. In addition, in situ analysis is usually final. That is, if questions are raised about the validity of a measurement after the analysis, no sample is available for verification.

46. To minimize the risk involved in relying on either method alone, a combination of the two may be employed--a preliminary in situ analysis to help guide the sampling effort and a final laboratory analysis to ensure accurate results for quantitative analysis.

47. Regardless of when and where fluorometric analysis takes place, several general precautionary measures should be taken to ensure that the analysis is reliable.

  a. The fluorometer should be accurately calibrated.

  b. Sample contamination should be avoided by rinsing or flushing the sample chamber between readings.

  c. The fluorometer operator should have experience with the instrument that is used. Experience can be gained through practice prior to the analysis.

<u>d</u>. Sample temperatures should be observed and recorded during analysis to determine the necessary fluorescence correction factors.

<u>e</u>. All information used to determine concentration units should be recorded (i.e., scale and meter or dial deflection).

<u>f</u>. The calibration should be checked on a regular basis (every hour or so). This is especially important if the fluorometer is powered by a battery; when the battery is drained, readings are no longer accurate.

48. For flow-through analysis in particular, all connections between the sampling hose, fluorometer, and pump must be tight to prevent air bubbles from entering the sample chamber. Air bubbles may also be introduced by a leaky pump seal; thus, it is recommended that the pump be connected to the system so that water is drawn up through the fluorometer to the pump. A screen placed at the intake end of the sampling hose will prevent sand and pebbles from altering the optics of the system since they may scratch the glass in the sample chamber as they travel through the system.

49. When analyzing samples in cuvette mode, the optics of the system may be distorted by scratches or smudges on the cuvette, making it necessary to wipe the cuvette clean prior to its insertion in the sample chamber. Once the cuvette is inside the warm sample chamber, a reading must be made quickly to prevent warming of the sample or condensation forming on the cuvette. Warming of the sample would cause a reduction in fluorescence while condensation would distort the system optics.

PART V: DYE INJECTION

Instantaneous and Continuous Releases: An Overview

50. There are two types of dye releases that can be made into the reservoir inflow, instantaneous and continuous. The instantaneous or "slug" release is used for marking a small parcel of water and is usually used in time of travel and dispersion studies. An instantaneous release can be done by one person without any special equipment. The relatively small plume that is created by the release can easily be followed and sampled by a small crew. Immediately after the release, the dye is in a compact plume in relatively high concentrations, producing a spatial concentration distribution that resembles a sharp and narrow spike (Figure 4, $t = 0$). The distribution eventually takes on a skewed Gaussian (normal) shape (Figure 4, $t = 1, 2$) as longitudinal dispersion elongates the plume and reduces the concentrations within it.

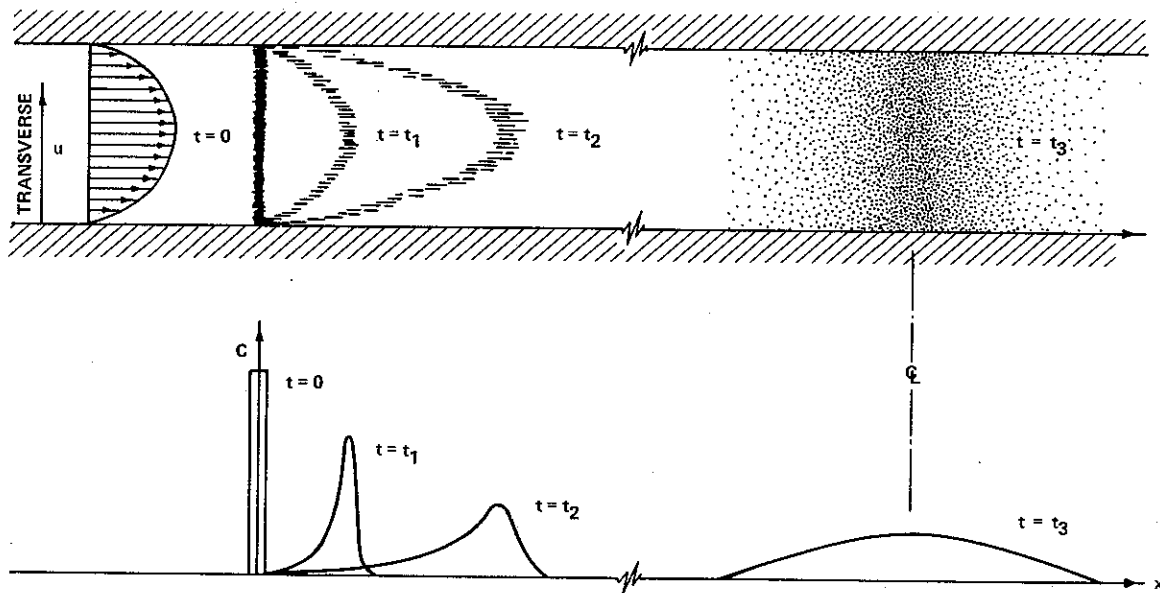51. The concentration distribution resulting after a continuous

Figure 4. Time variations of dye dispersing after a slug release (top);
spatial cross-sectionally averaged concentration distributions
corresponding to above snapshots (bottom)

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release resembles a Gaussian distribution with a plateau peak region (Figure 5). As the large plume is advected downstream, longitudinal
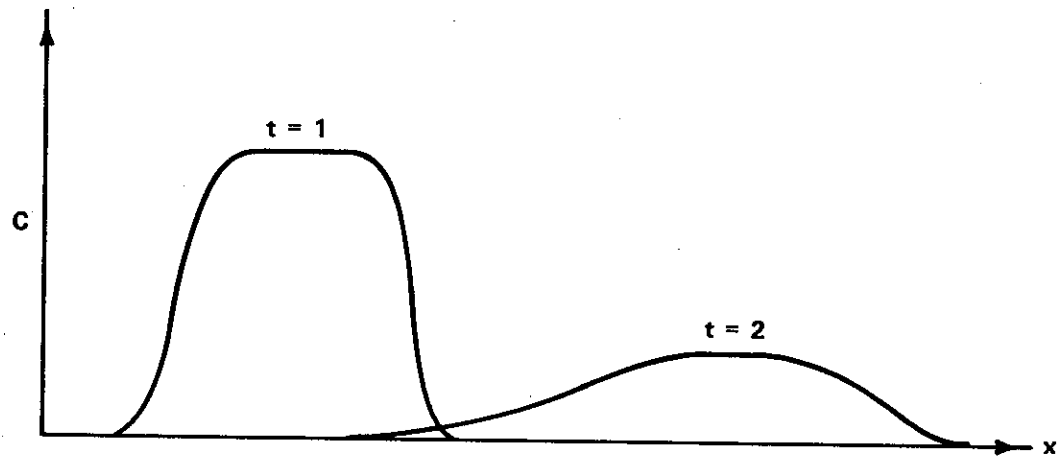

Figure 5. Cross-sectionally averaged spatial concentration distributions resulting after a continuous release

dispersion shortens the length of the plateau region while transverse and vertical mixing reduce the height of the plateau (i.e., the maximum concentration). Thus, continuous releases are used primarily for studying transverse and vertical mixing or when a large parcel of water must be marked. A large amount of dye is necessary for a continuous injection, along with a large field crew to sample the large dye plume it creates; both requirements make this an expensive method to implement. In addition, an accurate means of metering out the dye, such as a Mariotte vessel (Figure 6), is necessary. Pumps may also be used but are less desirable since variations in their delivery rate due to overheating or power fluctuations often occur.

Figure 6. Mariotte vessel (flow rate is a function of h)

Injection Site Location

52. To mark the inflow properly, the injection of dye should be made far enough upstream of the reservoir backwater to allow complete mixing of the dye vertically and laterally in the inflow prior to its entry into the reservoir. Any cross-sectional variations in concentration found in the inflow after it has entered the reservoir can then be attributed to mixing processes within the reservoir. In rivers, vertical mixing usually occurs more rapidly than lateral mixing; thus, the injection point location is usually determined by the distance required for complete lateral mixing, the initial mixing zone.

Background theory

53. During the early stage of the transport process after a dye release has been made, cross-sectional mixing is incomplete because of the dominating effects of transverse velocity gradients which cause particles of dye at different points in the cross section to be advected downstream at different rates. Transverse diffusion smooths out the resulting transverse concentration gradients. Longitudinal advective transport is much greater than transverse diffusion transport and causes cross-sectionally averaged spatial concentration distributions to be highly skewed (Figure 4, t = 1, 2) (Fischer 1966a). Eventually, transverse diffusion smooths out the cross-sectional concentration differences and the transport can be considered to be one-dimensional (i.e., the transport process can be modeled considering changes only in the longitudinal direction) (Fischer 1966b, Sayre 1968). This marks the end of what is often termed the initial or advective period and the beginning of the Taylor period (also known as the dispersive or Fickian period).

54. During the Taylor period, longitudinal gradients are smoothed by longitudinal diffusion and differential advective transport caused by transverse velocity gradients; as a result, the skewness observed in spatial concentration distributions begins to decrease. At a long time and distance later, the distribution becomes approximately Gaussian (Figure 4, t = 3) (Fischer et al. 1979).

Length of initial mixing zone

55. It is difficult to accurately determine the length of the initial mixing zone unless data from a previous study on the tributary of interest are available. Therefore, it is usually best to conduct a preliminary dye study to determine its length. A method for estimating the length of the initial mixing zone is given by Fischer et al. (1979) as:

$$x' = \frac{x\alpha u^* d}{\bar{u}W^2} \tag{2}$$

where

x' = dimensionless distance

x = distance required for complete lateral mixing (m)

α = stream geometry coefficient (dimensionless)

u^* = shear velocity (which can be approximated by $0.1\ \bar{u}$) (m/sec)

d = mean depth in the river reach (m)

$\bar{u}$ = mean cross-sectional velocity in the river reach being studied (m/sec)

W = mean cross-sectional width of the river reach (m)

to define three distinct zones for cross-sectionally averaged longitudinal spatial concentration distributions:

a. $x' < 0.4$: distributions are skewed; longitudinal advective transport is much greater than transverse diffusive transport

b. $0.4 \le x' < 1.0$: skewness in distribution decays; transverse diffusive transport smooths out cross-sectional concentration differences

c. $x' \ge 1.0$: distributions become approximately Gaussian

Thus, Equation 2 is solved for x after substituting in the value for x' that defines the length of the initial mixing zone (0.4), which yields:

$$x = 0.4\ \frac{\bar{u}W^2}{\alpha u^* d} \tag{3}$$

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56. It must be noted, however, that this relationship gives only an order of magnitude estimate for the length of the initial mixing zone. The stream geometry coefficient, α , is at best difficult to estimate. Fischer et al. (1979) state that, in general, $0.4 < \alpha < 0.8$ for slowly meandering streams but warn that when channel morphometry changes abruptly or sharp bends occur, α may increase beyond 0.8.

Effect of separation
zones in initial mixing zone

57. The initial mixing zone often extends beyond the distance predicted by Equation 3 in natural channels. Separation zones ("dead" zones or nonconveyance portions of the channel) trap and slowly re-release dye, causing sustained skewness in spatial concentration distributions (i.e., long "tails" in the upstream direction on distributions as shown in Figure 4, t = 1, 2) (Tsai and Holley 1979, Valentine and Wood 1977). Thus, dye dosages may need to be increased to compensate for the dye that may be trapped in separation zones.

58. The skewness observed in a spatial concentration distribution should not be confused with the skewness always found in temporal concentration distributions (paragraph 83). When the dye cloud is moving rapidly, it becomes impossible to determine a spatial distribution of the dye concentrations (where concentrations are measured instantaneously at a number of given locations) and a temporal distribution must be measured instead (i.e., concentrations must be measured through time at a fixed point). Unlike spatial distributions of dye concentration, a temporal distribution always appears skewed because of the continuous smoothing of longitudinal concentration gradients that occurs as the dye cloud passes the observation point (Holley and Harleman 1965).

Effect of injection point
position on initial mixing zone

59. The distance required for complete lateral mixing is also affected by the number and position of injection points in the river. If a single side discharge of dye is made, it can be shown from Equation 3 that the mixing distance is increased by a factor of 4 since the dye must travel twice the distance that it must travel for a center-line

injection to reach its boundaries (i.e., W^2 must be replaced by $(2W)^2$ = $4W^2$). Conversely, if a multipoint injection system is used, the mixing distance is reduced considerably. Specifically, the distance is reduced by a factor of n^2 when the injection point cross section is divided into n subsections of equal discharge and each subsection is dosed equally at its midpoint (i.e., in Equation 3, W^2 is replaced by $(W/n)^2$. When only one release point exists, therefore, it should be located at the half-discharge point.

Dosage Requirements for an Instantaneous Release

Empirical method

60. Once the injection site has been properly located, dye dosages must be calculated. Using data from 85 time of travel studies, Kilpatrick (1970) formulated the following relationship for an instantaneous release of Rhodamine WT dye:

$$V_d = (3 \times 10^{-5})C_p \frac{QL}{\bar{u}} \tag{4}$$

where

V_d = volume of dye (manufacturer's stock solution) required at injection (ℓ)

C_p = peak concentration desired at the point of interest (ppb)

Q = maximum discharge in the study reach (m^3/sec)

L = distance from the injection point to the point of interest (km)

$\bar{u}$ = mean velocity in the reach of length L (m/sec)

61. This relationship may be used to estimate the amount of Rhodamine WT dye needed to reach a given concentration at the beginning of the reservoir backwater. The concentration will depend on several factors including whether or not water intakes are located in the vicinity (limiting concentrations to less than 10 ppb) and the distance the inflow must be followed as it moves through the reservoir (the longer the distance, the higher the concentration required). Kilpatrick suggests

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that increased dosages might be warranted when lower velocities or ir-regular channels are encountered. Kilpatrick also warns that use of Equation 4 when values of $QL/\bar{u}$ exceed 6.7×10^5 may result in exces-sive dosages and suggests that the following equation be used instead:

$$V_d = (6 \times 10)^{-5} C_p \left(\frac{QL}{\bar{u}} \right)^{0.93} \tag{5}$$

Analytical method

62. If a dye other than Rhodamine WT is to be used or an alterna-tive method for determining dye dosage is desired for comparison to re-sults obtained using Kilpatrick's method, instantaneous release dosages may be estimated using a solution to the one-dimensional dispersion equation

$$\frac{\partial \bar{C}}{\partial t} + \bar{u} \frac{\partial \bar{C}}{\partial x} = D_L \frac{\partial^2 \bar{C}}{\partial x^2} \tag{6}$$

where

$\bar{C}$ = average cross-sectional concentration

D_L = longitudinal dispersion coefficient

$D_L \approx \dfrac{0.011 \bar{u}^2 w^2}{u^* d}$ (Fischer et al. 1979).

For the initial condition of a line source instantaneously distributed over the cross section and the boundary conditions of steady uniform flow in a channel of constant width and depth, the solution takes the form of a Gaussian distribution:

$$C(x,t) = \frac{M}{Wd\sqrt{4\pi D_L t}} \exp \left(- \frac{(x - \bar{u}t)^2}{4 D_L t} \right) \tag{7}$$

where

$C(x,t)$ = concentration at point x and time t $(g/m^3 = ppm)$

M = mass of dye instantaneously injected (g)

t = time elapsed since injection (sec)

x = distance from injection point to measuring point (m)

For the peak concentration at a given location $(x = \bar{u}t)$, Equation 7 can be reduced to:

$$C_{max}(x) = \frac{M}{Wd\sqrt{4\pi D_L \frac{x}{\bar{u}}}}$$

(8)

63. Equations 7 and 8 can be easily programmed on a calculator or computer to allow the computation of in-stream concentrations resulting from an instantaneous release of specified strength.

64. Since Fischer's numerical experiments (Fischer et al. 1979) showed that Gaussian spatial concentrations do not occur until approximately $x' > 1.0$, Equations 7 and 8 do not apply exactly until a distance at least 2.5 times larger than that given by Equation 3 ($x' > 1.0$ versus $x' > 0.4$). However, they may still be used for estimating concentrations downstream of the injection point (past the initial mixing zone) providing one realizes that the actual distribution will be skewed (as described in paragraph 53) and not normally distributed. An estimate is usually the best that can be done for most systems since detailed morphometric and mixing data are generally not available. The uncertainty may again warrant that a preliminary dye study be conducted. If more detailed information is available and a more accurate method is needed for determining concentration distributions resulting from an instantaneous release, the reader is referred to the techniques described by Fischer (1968) or Harden and Shen (1979).

Injection Rates for a Continuous Release

65. An approach similar to that used for estimating instantaneous release dosages can be taken when estimating the amount of dye needed for a continuous release. A solution of the dispersion equation using the method of superposition for a continuous release is given by Fischer et al. (1979) as:

$$C(x,y,y_o) = \left(\frac{\dot{M}}{\bar{u}d \sqrt{4\pi\varepsilon_t \frac{x}{\bar{u}}}} \right) * \sum_{n=-\infty}^{\infty} \left\{ \exp\left[-\frac{\left(\frac{y}{W} - 2n - \frac{y_o}{W}\right)^2}{\frac{4x\varepsilon_t}{\bar{u}W^2}} \right] \right.$$

$$\left. + \exp\left[-\frac{\left(\frac{y}{W} - 2n + \frac{y_o}{W}\right)^2}{\frac{4x\varepsilon_t}{\bar{u}W^2}} \right] \right\} \qquad (9)$$

where

C = concentration at position (x,y) for a given injection position, y_o (ppb)

y = lateral position in stream (m)

y_o = lateral position of source in stream (m)

$\dot{M}$ = rate of injection of dye (mg/sec)

ε_t = transverse mixing coefficient = $\alpha u * d$ (m^2/sec)

66. Equation 9 can also be easily programmed on a calculator or computer to allow the computation of concentrations downstream of a source of specified strength. A single hand calculation will help determine how many of the summation computations are necessary before they become insignificant; usually only a few are necessary (i.e., $n = -1$, 0, +1). For the peak concentration at a given location during a continuous release, Equation 9 reduces to

$$C_{max}(x) = \frac{\dot{M}}{\bar{u}d \sqrt{4\pi\varepsilon_t \frac{x}{\bar{u}}}} \qquad (10)$$

67. Equation 9 is applicable immediately downstream of the source since it describes both lateral and longitudinal concentration variations in the river but should only be used for approximating these concentrations since irregularities in natural channels cause deviations from the assumptions used in its derivation (uniform flow and constant velocity throughout the channel). This often gives the best estimate

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possible since knowledge of a river's morphometry and mixing character-
istics is usually limited. If, however, detailed information on the
river's morphometry and mixing characteristics is available and better
predictions are desired, the reader is referred to Yotsukura and Sayre's
(1976) description of the "cumulative discharge" method.

Reinjection

68. Sometimes it is desirable to reinject dye in an existing dye
cloud. Such is the case when only the location of a given inflow parcel
must be determined and concentrations within it become low. This situa-
tion may occur in time of travel studies and in studies of the type de-
scribed by Kennedy, Gunkel, and Carlile (1983) where a parcel of inflow
water is followed through a reservoir to monitor changes in its physical
and chemical characteristics.

69. The point at which dye is reinjected is usually determined by
locating the center of mass of the cloud; this requires that a three-
dimensional image of the dye cloud be obtained by sampling at various
points within it, a task which is usually a formidable one. The amount
of dye to be injected cannot be calculated using a simple formula under
such complex and dynamic conditions; rather, a judgment as to the amount
to use must be made based on such factors as how quickly the original
release of dye dissipated, whether or not water intakes are likely to be
passed, and how far the resulting new cloud of dye must be followed. It
is a difficult decision, since the amount of mixing that takes place may
change from what has occurred previously in response to changes in
reservoir geometry, meteorological conditions, and project operation.
Whenever possible, it is best to give the cloud as strong a dose as pos-
sible to reduce the risk of losing the plume because of diminished
concentrations.

70. There are some additional problems inherent in reinjecting
the plume in a stratified reservoir. The first of these is that the dye
solution that is injected may rise or sink from the level at which it is
introduced. Very small density differences (on the order of 10^{-3} kg/m^3)

can have a significant effect on the placement of the dye. If the inflow is moving through the reservoir as an interflow and the density of the injection solution is not adjusted to match the interflow's density, the dye solution may rise or sink to a level of neutral buoyancy outside the zone of flow. This would allow the inflow to pass by unmarked. Therefore, it is critical that the solution's density be adjusted prior to injection. This is particularly difficult since a 10:1 dilution of the dye in water only reduces its specific gravity to 1.12. In addition, temperature effects on its density must be considered. Thus, dilution water should be taken from the same level (i.e., similar density) to which the dye is to be introduced.

71. Another problem is that the momentum imparted at injection can affect the dye's placement in the pool. If the injection device creates a jet, the dye may be forced past its intended level in the pool. This is often a problem when a large volume of dye solution (created by performing the necessary dilution to minimize density effects) must be ejected quickly in a rapidly moving dye cloud. Thus, remarking an existing cloud can be an extremely difficult task.

Equipment

72. The basic equipment needed to perform an inflow field study
includes the following:

> a. Fluorometers and accessories (filters, spare lamps, re-
>   corders, and sample holders). A spare fluorometer should
>   be included if available since the entire field study
>   centers around its operation.
>
> b. Standard dye solutions for calibrating fluorometers.
>
> c. Generators or 12-volt deep-cycle marine batteries (with
>   charger) to power fluorometers and pumps.
>
> d. Sampling equipment--pump and hoses or discrete sampler
>   (e.g., a Van Dorn sampler), bottles, labels, waterproof
>   markers.
>
> e. Temperature-measuring device for measuring sample tem-
>   peratures and determining reservoir temperature profiles.
>
> f. Dye, dilution vessels, and injection equipment (e.g.,
>   Mariotte vessel (Figure 6), bucket, pump, and hoses).
>
> g. Maps or aerial photos of the study area and data forms.
>
> h. Position-fixing equipment (e.g., range finders, elec-
>   tronic distance-measuring devices, surveying equipment,
>   buoys).
>
> i. Boats and related equipment (e.g., motors, gas tanks,
>   life jackets, tool kits).

Additional equipment might include cameras, radios, and stream-gaging
equipment. All equipment should be checked for proper performance prior
to transporting to the field.

Preparatory Tasks

73. Once the equipment is ready for the field study, certain pre-
paratory tasks must be completed prior to the release of any dye. Since
inflow conditions (discharge and temperature) must be monitored con-
stantly during the study, existing gages should be checked for proper
operation. If none exist, new ones should be installed. The discharge
may need to be verified at the injection site if other inflows enter

between the injection site and the gage.

74. Bathymetric surveys should also be done at this time at various points along the study area in the reservoir. These are necessary if a mass balance analysis of the dye is to be conducted and may help in predicting how the inflow will travel through the reservoir. When vertical temperature profiles in the pool are measured prior to the release, and cross-section and inflow data are available, the techniques described in Ford and Johnson (1983) can be used to predict how the inflow will enter and move through the reservoir. This should eliminate much of the guesswork involved with the sampling effort in the reservoir.

75. One of the most important preparatory tasks is a preliminary site survey to determine if background fluorescence exists. Background fluorescence is defined as the sum of all contributions to fluorescence by materials other than the fluorescent tracer. Fortunately, little background fluorescence is found in the detection band for Rhodamine WT. Smart and Laidlaw (1977) concluded (contrary to popular belief) that algal pigments do not contribute significantly to background fluorescence in Rhodamine WT analyses. They pointed out that chlorophyll, the green pigment found in algae and other plants, fluoresces outside the Rhodamine WT detection band. However, red and blue-green algae contain accessory pigments (biliproteins) which do fluoresce. Phycoerythrin, the red pigment, fluoresces near the Rhodamine WT emission peak (Smart and Laidlaw 1977), which must be accounted for. Phycocyanin, the blue pigment, will cause no interference since it fluoresces outside the detection band for Rhodamine WT (Brock 1974).

76. Materials found in concentrated dissolved and colloidal organic matter may also cause background fluorescence in the Rhodamine WT detection band. Smart et al. (1976) found a correlation between total organic carbon concentrations and fluorescence over a wide band (including the detection band for Rhodamine WT) in several rivers in England. The fluorescence found in "sufficiently concentrated" dissolved and colloidal organic matter is due to "complex polymeric hydroxy-carboxylic and aromatic acids which frequently contain fluorescent structures" (Smart and Laidlaw 1977).

77. When background fluorescence is found, it must be determined if it is uniform throughout the study area. If it is, its contribution to the total fluorescence is simply subtracted from all subsequent fluorescence readings to determine the concentration of dye at any given location. When background fluorescence varies throughout the study area, the spatial variation must be determined; then when sampling occurs, the background value for a given zone can be referenced and subtracted from the total fluorescence measured to arrive at the true concentration of dye in a sample.

78. Another important item to be checked during the preliminary survey is whether or not turbidity levels are high enough to affect the analysis. When the water is very turbid, absorption or scattering of the light from the fluorometer lamp occurs, resulting in an overall reduction in fluorescence.

79. A simple test can be done to determine if turbidity levels are high enough to affect the sample fluorescence. First, a sample of the turbid water is divided in half and a small amount of dye is added to one of the portions. The fluorometer is blanked on the portion without dye in it and the fluorescence of the portion containing dye is measured. Next, both samples are filtered or centrifuged to eliminate the turbidity. The process is then repeated using the supernatants-- blanking the fluorometer on the portion without dye in it and measuring the fluorescence of the portion with dye in it. If the fluorescence value of the portion containing dye changes, then turbidity is indeed affecting the analysis. If turbidity is uniform throughout the study area, the ratio of the value obtained before eliminating the suspended materials to the value measured after doing so may be used as a correction factor that is applied to each fluorescence reading before it is converted to a concentration value. When turbidity levels vary widely throughout the study area, it becomes necessary to filter or centrifuge each sample or allow the suspended materials to settle out (prior to analysis).

Sampling Methods

Lagrangian approach

80. There are two methods by which to observe and sample the dye-marked inflow as it moves through the reservoir. In the first or Lagrangian approach (the approach used to obtain the spatial concentration distribution discussed in paragraphs 50 and 51), the observer moves with the dye cloud and samples such that three-dimensional "snapshots" are made at various times during its progression through the reservoir. The snapshot is produced by sampling at several depths and locations in lateral transects positioned throughout the length of the cloud.

81. In order to use the Lagrangian method, the sampling crew must be able to (a) complete the sampling necessary to make the "snapshot" in a period short enough to be considered "instantaneous" with respect to changes in the cloud's position and size, and (b) accurately record sampling locations on a map or aerial photograph for use during data analysis. If it takes too long to complete the sampling effort or sampling positions are inaccurately recorded, the snapshot is blurred or distorted and it becomes difficult to interpret the results of the sampling effort.

82. The amount of time necessary to sample the entire dye cloud depends on such factors as cloud size; lateral, vertical, and longitudinal concentration gradients within it; the manpower and amount of equipment available; and the type of study that is being conducted. When the cloud is large and concentration gradients within it are strong, many samples must be taken to describe it accurately. For studies in which only the determination of time of travel or vertical placement in the reservoir are important, detailed descriptions are not necessary and sampling may be completed quickly. When the sampling occurs in an expansive part of the reservoir or in a section void of landmarks, it becomes necessary to use special distance-measuring equipment (e.g., parallax rangefinders, transits, or electronic distance-measuring devices) to locate sampling positions accurately. If personnel or time constraints are such that sample positions cannot be measured when samples are taken, buoys may be dropped at each position and located later.

Eulerian approach

83. When the Lagrangian approach to observing and sampling the dye cloud cannot be implemented, the Eulerian approach must be used. With the Eulerian approach, the dye cloud is observed and sampled from fixed points in the reservoir. A time history or temporal distribution of concentration is recorded at each point.

84. When the dye is not well mixed vertically or laterally in the observation cross section, it becomes necessary to sample at more than one location in the observation transect. If the dye cloud moves very slowly, this method is a very inefficient method for collecting data since the equipment is idle while waiting for the cloud to pass one position when it could be used to sample at many positions in the reservoir.

85. One advantage of using this method is that automatic samplers can be used to reduce the manpower requirements for the study. Overall, the most appealing facet of this method is its inherent simplicity-- equipment and personnel must be moved only occasionally and only a few locations must be charted on a map or aerial photograph.

Photography as a Sampling Supplement

86. If the dye cloud is visible, photography may be used to supplement the sampling effort. Aerial photographs, in particular, can provide information on the spatial characteristics of the cloud that an intensive sampling effort might not furnish. Color films that are especially sensitive to blue wavelengths (e.g., Kodak Ektachrome) allow the dye to be seen easily since these wavelengths penetrate the water column best. Black-and-white panchromatic films can also be used to photograph the dye. Best results are obtained with color films, however. When black-and-white films are used in conjunction with an orange, red, or deep red filter (e.g., Wratten Series 15(G), 23A, and 25 or Corning 3-66), the dye cloud appears as a white image against the dark-colored ambient water (Wilson 1968).

PART VII: DATA ANALYSIS

87. Since data collection is influenced by study objectives and study objectives will vary according to the problems being addressed, only a general discussion of data analysis is possible. There are a few steps in analyzing the data that are common to all studies.

Conversion of Fluorescence Readings to Concentration Units and Determination of Dye Recovery

88. First, all fluorescence readings must be properly converted to concentration units. This means that all readings must be corrected for effects of such influences as temperature and turbidity, as discussed in paragraphs 17 and 79.

89. The second step in analyzing the data is to determine how much of the dye was recovered (measured) downstream at different sampling locations. If a large portion (i.e., greater than 10 percent) of the injected dye mass cannot be accounted for, it is difficult to defend any type of quantitative analysis that might be performed. A high percentage recovery of dye, however, is usually indicative of good field technique.

90. The method used to determine the percentage recovery of dye depends on which sampling approach is used.

91. When the Eulerian approach is used, the concentration-versus-time curve for a given location is first integrated; then the results of the integration are multiplied by the flow rate to yield the mass of dye which passed. This approach assumes steady flow; if unsteady flow occurred, the product of incremental time, average concentration, and average flow rate for discrete intervals of time must be summed over the entire sampling period to determine the mass of dye which passed.

92. When the Lagrangian approach is used to sample the dye, the mass of dye in the plume at a given time can be estimated by: (a) breaking the plume into subsections, (b) determining the average concentration in each subsection, (c) multiplying the average concentration within a subsection by the volume within it, and (d) summing these values to

obtain the total mass within the plume. Theoretically, the smaller the
subsections used in this type of analysis, the more accurate the results.
In reality, though, the spatial distribution of the data is usually too
sparse to permit such partitioning. One difficulty with using this
method, therefore, is that the error introduced in averaging the concen-
trations and estimating the values may exceed the losses which actually
occurred during the study.

Interpretation

93. When interpreting the data from an inflow study, it is of ut-
most importance to keep in mind the assumptions and limitations of the
methods employed. If, for example, background fluorescence was assumed
constant but in fact varied, a mass balance might show a dye recovery
substantially larger (or smaller) than 100 percent. Other assumptions
and limitations which might influence a study include: (a) the limita-
tion imposed by the large specific gravity of the dye (the dye may sink,
even after much dilution); (b) the assumption that the dye is completely
mixed laterally and vertically in the inflow prior to its entry into the
reservoir; and (c) the limitation imposed by the amount of data that was
able to be collected (imperfect knowledge).

94. It is also important to consider the conditions under which
the study took place (i.e., stratification, flow, meteorology, project
operation, etc.). Ford and Johnson (1980) found that a large plume
created by a continuous release of Rhodamine WT separated into three
distinct parts (Figure 7). This breakup was discovered to be due to the
effects of convective cooling. The plume passed over two deep pools
during the evening when the surface waters, cooled by the October night-
time air, increased in density and mixed downward. Since a greater vol-
ume of water was available for mixing in the deep pools, concentrations
were reduced more than in the shallower portions of this region. Thus,
in this case the effects of meteorology, morphometry, and stratification
all had to be considered when interpreting the results.
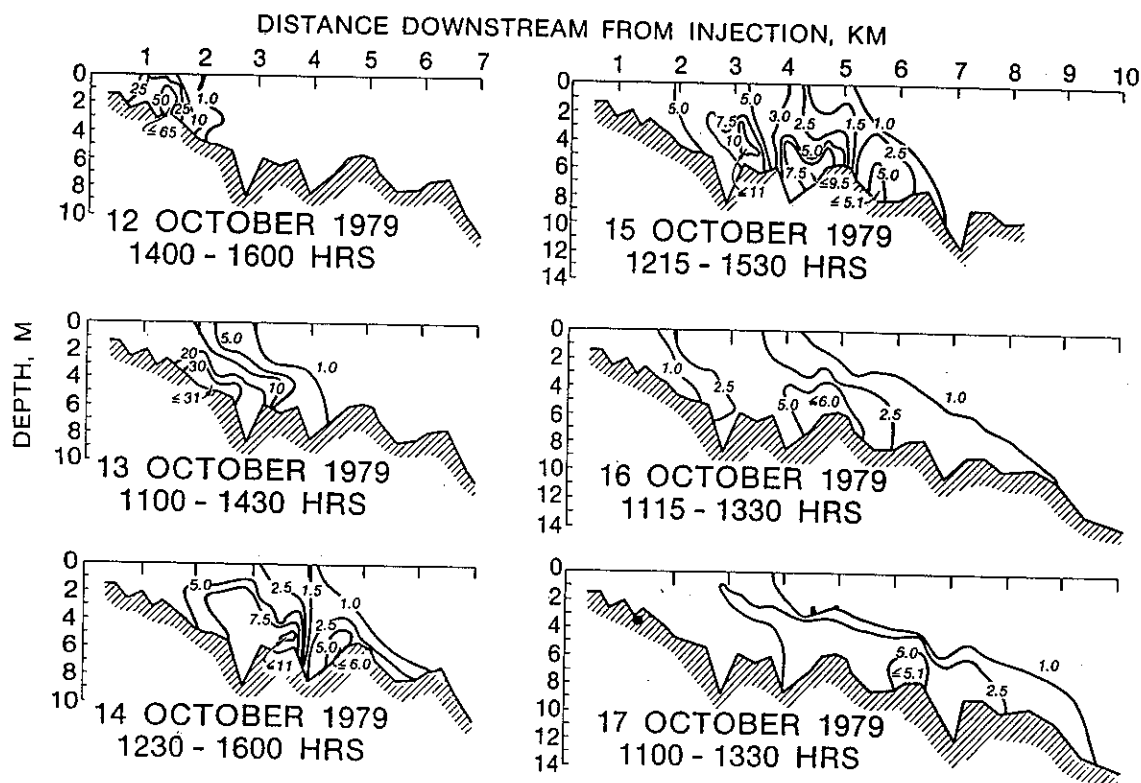
DISTANCE DOWNSTREAM FROM INJECTION, KM

Figure 7. Two-dimensional plots of dye isopleths for the inflow dye study described by Ford and Johnson (1980) (concentrations in ppb)

95. References which might give some insight into the analysis of inflow dye study data include:

a. Ford and Johnson (1983)--A paper on reservoir inflow processes.

b. Fischer and Smith (1983)--An investigation of dye exchange between surface waters and a plunging inflow to Lake Mead.

c. Kennedy, Gunkel, and Carlile (1983)--A study of the chemical and physical changes that occurred in a parcel of water as it moved through and mixed within West Point Lake, Georgia.

d. Sutron Corporation (1980)--An investigation of mixing of the Missouri River inflow in Lewis and Clark Lake, Missouri. Data from this study were used to verify a transport model.

e. Bayne (1967)--An investigation of the passage of the Chattahoochie River inflow through Lake Eufala, Georgia.

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96. One of the most valuable aids in interpreting the data is graphic presentation. With data collected via the Eulerian approach, the changing size and shape of the time-history curves show the effects of mixing and dispersion on the dye cloud (Figures 4 and 5). With data collected in a Lagrangian sampling effort, two-dimensional isopleth drawings (Figure 7) and three-dimensional representations can be made (Figure 8). A series of these plots allows the interpreter to quickly determine changes in the cloud's size and concentrations.
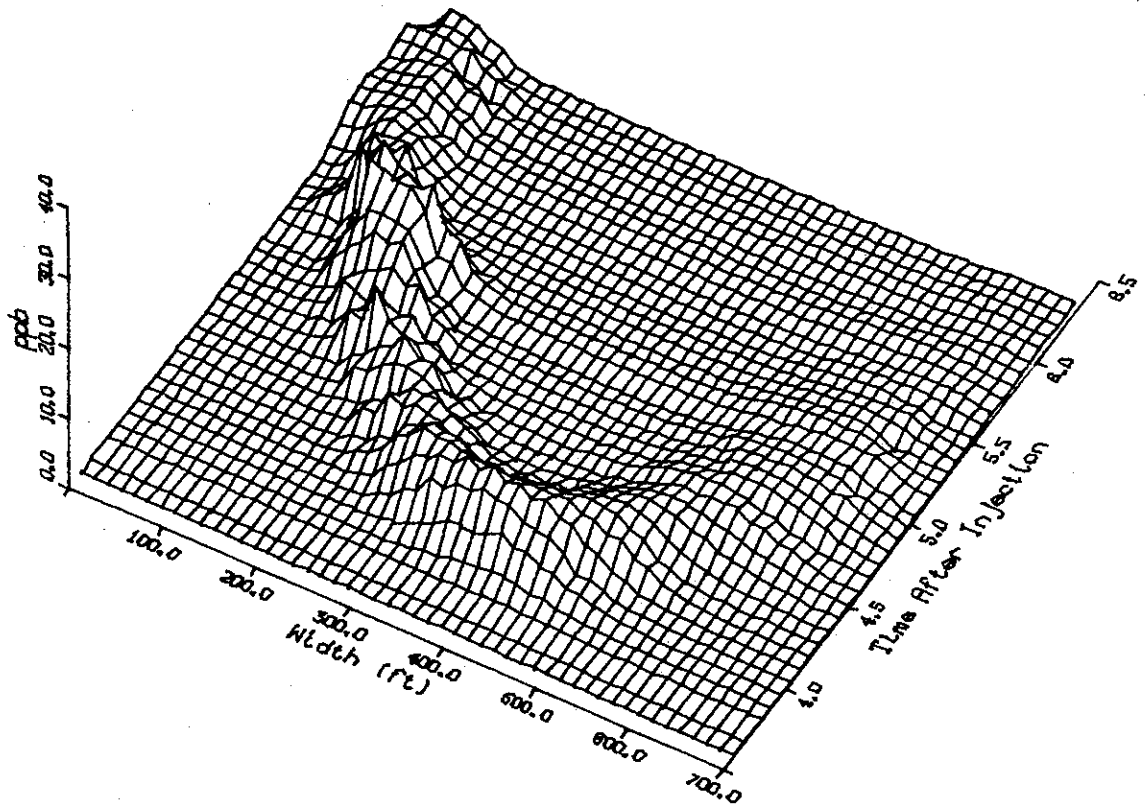

Figure 8.   Time history of dye concentrations passing an observation point presented as a three-dimensional plot (from Environmental Laboratory, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss., unpublished data)

PART VIII: SUMMARY

97. It is often important to know more than just how much of a given constituent enters a reservoir. It is important to also know how an inflow enters, moves through, and mixes within a reservoir. A reliable means of investigating this behavior has been through the implementation of an inflow dye study. When the inflow is marked with a fluorescent dye such as Rhodamine WT, the movement and mixing of the inflow in the pool can be quantified using fluorometric analysis.

98. Rhodamine WT has proven to be an easy and reliable tracer for use in inflow studies. It is a fairly conservative tracer that poses no known environmental or health hazards when used in unpolluted waters. An alternative dye, lissamine FF, does have certain advantages over Rhodamine WT as a tracer but, overall, is less desirable.

99. Although the principles of operation remain the same, recent advances in electronics and optics have made field fluorometry simpler than ever. The Turner Designs Model 10 Series fluorometer has replaced the Turner Model 111 fluorometer as the instrument preferred for field-work due to its higher sensitivity, easier calibration, ease in sensitivity selection, and its ability to operate from a variety of power sources.

100. Perhaps the most crucial part of the study is the injection of the dye. In order to study the mixing between the inflow and reservoir pool, the dye must be introduced at a location far enough upstream of the reservoir backwater to permit complete lateral and vertical mixing prior to its entry into the pool. In rivers, the distance required for complete lateral mixing is usually the limiting case, a distance which can be estimated using Equation 3. In most cases, dilution is required to prevent sinking of the dense dye.

101. If a small plume is desired, such as in the case in time of travel and dispersion studies, a slug injection of dye is used. Dosing requirements (and resulting downstream concentrations) can be estimated using Kilpatrick's empirical relationships (Equations 4 and 5) or a solution to the one-dimensional dispersion equation (Equations 7 and 8),

provided that certain assumptions used in its derivation are borne in mind.

102. When a much larger plume is desired, as is required when the dye must be followed for long distances or when lateral or vertical mixing is to be studied, a continuous release of dye must be made. Dosing requirements for a continuous release can be estimated using Equations 9 and 10.

103. When sampling the dye plume that is created from either method of injection, a Eulerian or Lagrangian approach may be used. Usually the choice of method is dictated by the study objectives and such factors as the size of the plume and the velocity at which it travels, and the manpower and equipment available.

104. When analyzing the data collected in the field, previous studies have shown that it is important to keep in mind the assumptions made and the limitations inherent in the techniques used. In addition, it is also important to consider the conditions under which the study was conducted since each set of conditions is unique and can influence the behavior of the inflow.

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